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Complete genome sequence of *Citrobacter freundii* 705SK3, an OXA-48 encoding wastewater isolate

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Abstract: We present the genome sequence of *Citrobacter freundii* 705SK3, a wastewater isolate harboring an IncL OXA-48-encoding plasmid. Assembly of the genome resulted in a 5,242,839-bp circular chromosome (GC content, 52%) and two closed plasmids of 296,175 bp and 63, 458 bp in size.

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**Complete genome sequence of *Citrobacter freundii* 705SK3, an OXA-48 encoding
wastewater isolate**

Genome of OXA-48 encoding *Citrobacter freundii* 705SK3

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21 **Abstract.** We present the genome of *Citrobacter freundii* 705SK3, a wastewater isolate,
22 harboring an IncL OXA-48 encoding plasmid. Assembly of the genome resulted in a
23 5,242,839 bp circular chromosome (GC content 52 %), and two closed plasmids of
24 296,175 bp and 63, 458 bp in size.

Citrobacter freundii is an opportunistic pathogen and is frequently found in the environment (water, soil), but can also be isolated from food or the intestines of animals and humans. Although its virulence potential is rather low, *C. freundii* can be the causative agent of a wide spectrum of infections involving the gastrointestinal, urinary, or respiratory tract or even the central nervous system (1). *C. freundii* possesses an inducible AmpC β -lactamase which can be a challenge for antibiotic susceptibility reporting, meaning an *in vitro* susceptibility may not correlate with clinical efficacy (2). With the general increasing number of carbapenemase-producing Enterobacteriaceae, also the reports of carbapenemase-producing *C. freundii* has increased (3-6). The main carbapenemases produced by Enterobacteriaceae belong to the Ambler class A (e.g. KPC), B (e.g. IMP, VIM and NDM) or the class D (e.g. OXA-48 and its variants). The latter generally possess weak but significant carbapenemase activity (7). However, the combination of an (inducible) AmpC β -lactamase together with an OXA-48 makes such isolates resistant against almost all β -lactams available.

Here, we present the genome of an OXA-48 producing *C. freundii* isolated from wastewater near Basel, Switzerland in December 2015 (8). The genome was sequenced at the Functional Genomics Center Zurich (FGCZ) using Pacific Biosciences (PacBio) single-molecule real-time (SMRT) technology RS2 reads (C4/P6 chemistry). *De novo* assembly was performed using SMRTAnalysis 2.3 with the HGAP3 protocol. Annotation was done using the NCBI Prokaryotic Genome Annotation Pipeline (9). MLST-1.8 Server (10), ResFinder 2.1 (11), and PlasmidFinder 1.3 (12) were used to identify sequence type (ST), acquired resistance genes and plasmid incompatibility types.

The assembly resulted in one chromosome and two plasmids (all sequences were

closed). The chromosome is 5,242,839 bp in size (GC content 52%) and encodes the AmpC β -lactamase CMY-75. The isolate could not be assigned to an existing ST, although it is highly similar to ST112 (only one point mutation in the *fadD* gene: allele 69 position 363G→A). The larger of the two plasmids, p705SK3_1, is 296,175 bp in size with a GC content of 47.8% and does not encode any antimicrobial resistance genes. PlasmidFinder was not able to assign it to any plasmid incompatibility group. The second plasmid (63,458 bp, GC content 51.2%), p705SK3_2, belongs to the incompatibility type IncL and carries the *bla*_{OXA-48} gene. It shows remarkable similarities to the prototype of the IncL plasmids involved in the worldwide spread of OXA-48 (7, 13). The main difference were two IS/R elements present on p705SK3_2 compared to pOXA-48 (JN626286). The first IS/R is located 163 bp downstream of the *bla*_{OXA-48} gene and the second one is 380 bp downstream of *korC*, which encodes a hypothetical transcriptional repressor. Furthermore, p705SK3_2 possesses 99.96% identity to p704SK10_2 (CP022150), an OXA-48 encoding IncL plasmid extracted from an *Enterobacter cloacae* isolate from the same wastewater (14).

The isolate *C. freundii* 705SK3 is a further proof that *Citrobacter* species have to be considered as potential reservoir for the wide disseminated OXA-48 encoding IncL plasmids.

Nucleotide sequence accession numbers. Sequence and annotation data of the genome have been deposited at GenBank under accession numbers CP022151 (chromosome), CP022152 (p705SK3_1) and CP022153 (p705SK3_2). This is the first version of this genome.

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